Forum Minireview

Progress in Allergy Signal Research on Mast Cells: Up-Regulation of Histamine Signal-Related Gene Expression in Allergy Model Rats

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Abstract. Brown Norway allergy model rats sensitized to toluene 2,4-diisocyanate (TDI) were developed. Histamine H1 receptor mRNA level was elevated in nasal mucosa of allergy model rats after the provocation with TDI, which was followed by H1-receptor up-regulation. Elevation of histamine H1 receptor mRNA was partially suppressed by d-chlorpheniramine and olopatadine, antihistamines. Histamine induced increase in histamine H1 receptor gene expression in vitro, and the protein kinase C-δ isoform was suggested to mediate the gene expression. On the other hand, elevation of histamine H1 receptor mRNA was completely suppressed by dexamethasone in allergy model rats. Provocation with TDI also induced mRNA elevation of histidine decarboxylase, a sole histamine-forming enzyme, followed by the increase of both HDC activity and histamine content in nasal mucosa of allergy model rats. HDC mRNA elevation and increase in both HDC activity and histamine level were almost completely suppressed by dexamethasone. These observations suggest that histamine H1 receptor up-regulation and increase in histamine level play an important role in allergy through the regulation of histamine signaling.

Keywords: allergic disease, histamine H1 receptor, histidine decarboxylase, antihistamine, dexamethasone, allergy

Introduction

Histamine is the principal mediator in allergy. Symptoms of allergy are supposed to be greatly influenced by changing the extent of histamine signaling. Histamine signaling is regulated either by expression level of histamine H1 receptors (H1Rs) or by histamine level synthesized by histidine decarboxylase (HDC). Elevation of H1R mRNA was observed in nasal mucosa of allergic rhinitis patients (1, 2) and in primary cultured cells from nasal mucosa of allergic rhinitis patients (3). Elevation of HDC mRNA was also observed in nasal mucosa of allergic rhinitis patients (4). Elevations of H1R mRNA and HDC mRNA are supposed to be followed by the up-regulation of H1Rs and increase in HDC activity as well as histamine level, respectively, leading worsening of allergy symptoms.

In order to elucidate patho-physiological mechanisms of allergic diseases, studies using allergy model animals are indispensable. Various allergy model animals with increased IgE level have been developed (5, 6). These models are beneficial because increased IgE signaling is quite natural in allergy. Their manipulations, however, are so complicated that these are not suitable for studies at the molecular level. So a simple and reproducible animal model of allergic disease was highly required. Toluene 2,4-diisocyanate (TDI), an ingredient of plastic, is suspected as a causative reagent for occupational asthma in plastic factories (7). Previously we developed allergic pathogenesis in guinea pigs (8, 9) and Brown Norway rats (10, 11), and studies of allergy at the molecular level have been achieved using these animal models.

In the present mini-review, increase in gene expression of H1R and HDC is described using Brown Norway allergy model rats. Suppression of H1R and HDC gene
expression by dexamethasone and antihistamines is also described as targets of therapeutics for allergic diseases.

Methods

Preparation of allergy model rats and assessment of nasal allergy behaviors

Six-week-old male Brown Norway rats were sensitized with TDI (Wako Chemical Co., Tokyo) (Fig. 1A) as described previously (10, 11). Briefly, 10 µl of a 10% solution of TDI in ethyl acetate was painted bilaterally on the nasal vestibules once a day for five consecutive days (Fig. 1B). This sensitization procedure was then repeated after a 2-day interval. Nine days after the second sensitization, provocation was performed by applying 10 µl of 10% TDI solution to the nasal vestibules. Nasal allergy behaviors were assessed using symptom scores as described previously (8). Briefly, the scores were assessed by number of sneezes and extent of watery rhinorrhea on a scale ranging from zero to three during a 10-min period just after TDI provocation (Table 1). Animal experiments were performed as per guidelines approved by the Ethical Committee for Animal Studies, School of Medicine, The University of Tokushima.

Pretreatment with antihistamines and dexamethasone

Both d-chlorpheniramine and olopatadine were administered orally 1 h before TDI provocation at a dose of 30 mg/kg, and pretreatment of dexamethasone was performed intraperitoneally 24 h before TDI provocation at a dose of 1.0 mg/kg.

Determination of H1R mRNA and HDC mRNA by real-time quantitative reverse transcriptase polymerase chain reaction (real-time PCR)

H1R mRNA and HDC mRNA were determined as described previously (10, 11). Briefly, total RNA was isolated from rat nasal mucosa using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA, USA). RNA samples were reverse-transcribed to cDNA. TaqMan primers and probe were designed using Primer Express software (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The sequences of the primers and probes have been stated in Table 2. The transcripts were utilized for a 40-cycle 3-step PCR using the GeneAmp 5700 Sequence Detection System (Perkin Elmer Applied Biosystems). For the quantification of gene expression, GAPDH mRNA was used as an endogenous control.

Table 1. Nasal score criteria of Brown-Norway allergy model rat sensitized with toluene 2,4-diisocyanate (TDI)

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sneezing</td>
<td>(-)</td>
<td>1–4</td>
<td>5–11</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Watery rhinorrhea</td>
<td>(-)</td>
<td>at the nostril</td>
<td>intermediate</td>
<td>dropping discharge</td>
</tr>
</tbody>
</table>

Nasal scores were assessed by counting the above-mentioned two parameters for 10 min just after provocation with TDI.

Table 2. Primer and probe sequences used for real-time RT-PCR

<table>
<thead>
<tr>
<th>Primer/probe name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1R mRNA</td>
<td>Sense primer</td>
</tr>
<tr>
<td></td>
<td>Anti sense primer</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
</tr>
<tr>
<td>HDC mRNA</td>
<td>Sense primer</td>
</tr>
<tr>
<td></td>
<td>Anti sense primer</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
</tr>
</tbody>
</table>
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Radioligand binding assay

H1R was determined by [3H]mepyramine (NEN Life Science Products, Boston, MA, USA) binding assay as described previously (10). In brief, nasal mucosa was homogenized in 10 volumes of ice-cold 50 mM Na2/K-phosphate buffer (37.8 mM Na2HPO4, 12.2 mM KH2PO4, pH 7.4) and then centrifuged at 1800 rpm for 30 min at 4°C. The pellet was resuspended in ice-cold 50 mM Na2/K-phosphate buffer and served as membrane sample for radioligand binding assay. Membranes were incubated with 4 nM of [3H]mepyramine in the absence or presence of 10 µM triprolidine for 60 min at 25°C in a final volume of 500 µl. Reaction was terminated by rapid vacuum filtration through Whatman GF/B filters (Maidstone, UK) presoaked with 1% polyethyleneimine. Filters were then soaked in 10 ml of Aquasol-2 (Packard Instrument Inc., Meriden, CT, USA), kept overnight in a dark place, and the radioactivity trapped on the filters was counted in a liquid scintillation counter. Specific binding was defined as the radioactivity bound after subtraction of non-specific binding as defined by 10 µM triprolidine. Protein concentration was determined by the BCA protein assay reagent (Sigma, St. Louis, MO, USA) using BSA as a standard.

H1R promoter assay

HeLa cells cultured in 12-well culture plates were co-transfected with Human H1R reporter plasmid (pH1R) and the internal control plasmid pRL-MPK containing cDNA encoding mouse pyruvate kinase and Rluc (Promega, Madison, WI, USA) by the ratio of 100:1 using PolyFect transfection reagent (Qiagen, Tokyo) according to manufacturer’s instructions. After 5 h, the medium was replaced with 1 ml of serum free medium and starved for 24 h. The cells were stimulated with appropriate reagents for the indicated time in the same medium. Where appropriate, antagonists were added 30 min prior to histamine stimulation. After stimulation, cells were washed twice with 500 µl of ice-cold phosphate-buffered saline and lysed with 100 µl of passive lysis buffer (Promega). The lysate was frozen for at least 3 h at −85°C and then thawed at room temperature. The luciferase activity was determined by the dual-luciferase reporter assay system (Promega) as per the manufacturer’s protocol. Luminescence was measured by photoluminescence reader BLR 302 (Aloka, Tokyo). The measurement was integrated over 20 s with no delay.

Determination of HDC activity and histamine content

Samples for the determination of HDC activity and histamine content were prepared by the method of Yamada et al. (12). HDC activity was determined by the method of Watanabe et al. (13). Histamine content was measured using a high-performance liquid chromatography with a cation exchanger and an automated o-phthalaldehyde fluorometric detection system in accordance with the method of Yamatodani et al. (14).

Statistical analyses

The results are presented as mean ± S.E.M. All P values were determined by using one-way ANOVA and Fisher’s paired least-significant difference test. P values less than 0.01 were considered significant.

Results

Increase in allergic symptom after provocation and their suppression by therapeutics for allergy

Severe nasal swelling and watery rhinorrhea was observed in Brown Norway allergy model rat after the provocation with TDI (Fig. 2, right panel). The number of sneezes was also highly increased. Nasal scores based on number of sneezes and watery rhinorrhea increased remarkably compared with the control animals (Fig. 3).

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Results

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Severe nasal swelling and watery rhinorrhea was observed in Brown Norway allergy model rat after the provocation with TDI (Fig. 2, right panel). The number of sneezes was also highly increased. Nasal scores based on number of sneezes and watery rhinorrhea increased remarkably compared with the control animals (Fig. 3).

Fig. 2. Nasal swelling and rhinorrhea of Brown-Norway allergy model rat after provocation with 10% toluene 2,4-diisocyanate. Control (left panel) and allergy model rat (right panel).

Fig. 3. Increase in nasal score of Brown-Norway allergy model rats after provocation. Score was assessed for 10 min just after provocation according to Table 1. Data each represent a mean ± S.E.M. **P<0.01, n = 4.
Increase in nasal score was significantly suppressed by the administration of \(d\)-chlorpheniramine prior to TDI provocation (Table 3). Although pretreatment of dexamethasone also suppressed the elevation of symptom score, the extent of suppression was smaller than \(d\)-chlorpheniramine. The score assessment was achieved for 10 min after the provocation, and the blockade by antihistamine of H1R signaling seemed to suppress the symptom more strongly.

**Elevation of H1R mRNA and H1R protein level**

Elevation of H1R mRNA was induced after the provocation with TDI (Fig. 4A), and this led to H1R up-regulation (Fig. 4B). It is well known that H1R is desensitized after repetitive stimulation of H1R, and down-regulation of H1R is induced as a final step of desensitization (15 – 17). Therefore expression level of H1R is the result of up-regulation due to H1R gene expression and down-regulation due to the desensitization. H1R up-regulation actually overcame the down-regulation in Brown Norway allergy model rats.

H1R mRNA up-regulation was partially but significantly suppressed by \(d\)-chlorpheniramine and olopata- dine, antihistamines, in allergy model rats (Fig. 5). This data suggested that H1R mRNA up-regulation is mediated through H1R signaling and some other factors may also be involved in this up-regulation. We previously demonstrated the H1R-mediated elevation of H1R mRNA in HeLa cells. As shown in Fig. 6A, Ro-31-8220, a nonspecific PKC inhibitor, completely inhibited histamine-induced up-regulation of H1R gene expression. Similar results were obtained in the H1R promoter assay also (Fig. 6B). These data indicate that the PKC pathway is involved in H1R-mediated H1R gene expression. Many PKC isoforms have been reported. Some are involved in desensitization of various receptors. The PKC-\(\alpha\) isoform was reported to engage desensitization of \(\alpha_1\)-adrenoceptors (18) and \(\mu\)-opioid receptors (19).

**Table 3.** \(d\)-Chlorpheniramine and dexamethasone suppress nasal symptoms in allergy model rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TDI control</th>
<th>TDI + (d)-chlorpheniramine</th>
<th>TDI + dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal score</td>
<td>0</td>
<td>5.0 ± 0.3</td>
<td>2.2 ± 0.4**</td>
<td>3.5 ± 0.5**</td>
</tr>
</tbody>
</table>

The values are the mean ± S.E.M. **P<0.01 vs TDI-sensitized group.

**Fig. 4.** Elevation of histamine H1 receptor mRNA (A) and H1 receptor up-regulation (B) in nasal mucosa of Brown-Norway allergy model rats after provocation. In panel A, mRNA levels after provocation and control are represented with closed squares and closed circles, respectively. Data each represent a mean ± S.E.M. **P<0.01 vs control, n = 4.

**Fig. 5.** Suppression of histamine H1 receptor mRNA elevation by antihistamines (\(d\)-chlorpheniramine: dCPh, 30 mg/kg, i.p.; olopata- dine: Olo, 30 mg/kg, i.p.) and dexamethasone (Dex, 1 mg/kg i.p.) in nasal mucosa of Brown-Norway allergy model rats after provocation. Data each represent a mean ± S.E.M. **P<0.01 vs control, n = 4 and ***P<0.01 vs none, n = 4.
isoform completely suppressed H1R-mediated H1R gene expression (unpublished data). PKC-α and PKC-δ isoforms seem to exert opposite regulatory functions in receptor signaling. On the other hand, H1R up-regulation was completely suppressed by dexamethasone (Fig. 5). There are many reports about suppression of various gene expressions by dexamethasone (20, 21). Genes related to inflammation seem to be suppressed by dexamethasone. Dexamethasone did not show any strong advantageous effect on symptoms just after TDI provocation (Table 2), but thought to induce long-term suppression of H1R signaling through suppression of H1R expression.

Dexamethasone suppresses TDI-induced elevation of HDC mRNA, HDC activity and histamine level in allergic rats

Elevation of HDC mRNA was also induced in nasal mucosa of Brown Norway allergy model rats after provocation with TDI (Fig. 7A). Increase in HDC activity and histamine level also followed (Fig. 7B and C). Increase in histamine level in nasal mucosa is thought to increase histamine signaling and worsen allergy symptoms. Elevation of HDC mRNA, HDC activity, and histamine level were all suppressed strongly by dexamethasone (Fig. 8). Long-term suppression of histamine signaling seems be exerted through...
lowering not only the H1R expression level but also the histamine level.

**Concluding remarks**

Levels of H1R mRNA and HDC mRNA were highly elevated in nasal mucosa of Brown Norway allergy model rats after the provocation with TDI (Figs. 4A and 8A). Up-regulation of H1R and increase in HDC activity and histamine level also followed these increases (Figs. 4B, 8B, and 8C). Increase in gene expression of H1R and HDC is suggested to play an important role in increasing histamine signaling and worsening allergy symptoms (Fig. 9). Antihistamines are used to suppress immediate allergy symptoms by blocking H1R signaling. Although the effect was partial, antihistamines also suppressed H1R mRNA elevation. The data indicate that the therapeutic effect of antihistamines is long lasting. Dexamethasone completely suppressed elevation of both H1R mRNA and HDC mRNA. Pretreatment of dexamethasone could maintain H1R and histamine at lower levels. The effect of dexamethasone on histamine signal-related gene expression is thought long lasting, compared with antihistamines.

Only little attention has been paid to the long-term increase in histamine signaling through increase in histamine signal-related gene expression. To my best knowledge, no attention has been paid to development of new therapeutics for allergy by suppressing gene expression of H1R and HDC. Antihistamines actually showed some effect on H1R gene expression (Fig. 5). Although dexamethasone showed a strong effect on the two key genes (Figs. 5 and 8), use of this compound is limited due to the side effect. Therefore other medicines possessing the suppressing effect on elevation of H1R mRNA and HDC mRNA with less side-effect is worth developing. Elevation of H1R mRNA was strongly suppressed by suplatast tosilate and other natural source medicines including Sho-seiryu-to (A. Das et al., unpublished data) and Kujin (Sophora flavescens) (S. Dev et al., unpublished data). Long-term treatment of antihistamines also showed potent suppressing effect on H1R gene expression (M. Hatano et al., unpublished data). In the case of HDC gene expression, some antihistamines revealed to possess strong suppressing effects (A. Das et al., unpublished data). Although the mechanism of HDC gene expression is complicated and has not been extensively investigated, the molecular target of some
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antihistamines remains to be elucidated. Finally, targets of these medicines are quite different. On the other hand each allergic patient could show different patterns of H1R gene expression and HDC gene expression. Thus, a therapeutic method for each medicine should be developed as a tailor-made therapy.

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References